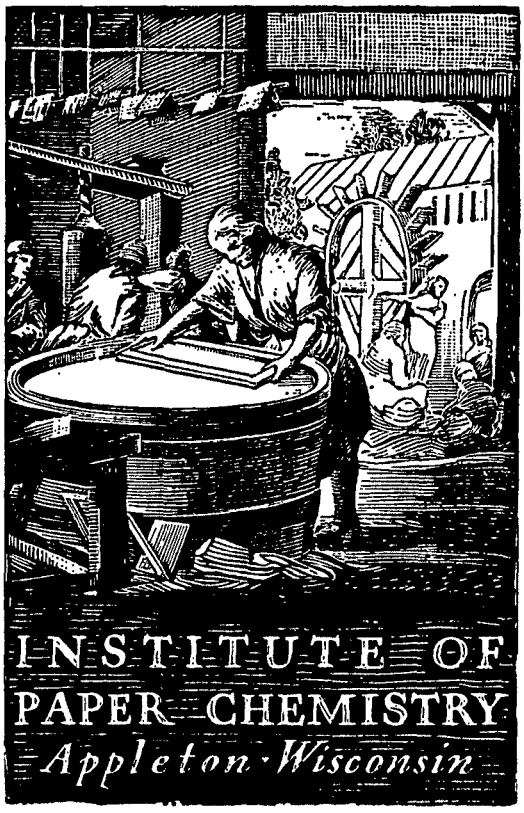

STUDY OF THE CARBOHYDRATE PEELING AND STOPPING REACTIONS UNDER THE CONDITIONS OF OXYGEN-ALKALI PULPING

Project 3265

Report Four
Final Report
to
MEMBERS OF THE INSTITUTE OF PAPER CHEMISTRY

March 1, 1979

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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SUMMARY

The purpose of this project was to determine the feasibility of conferring alkali stability to carbohydrate polymers by oxidizing their end groups to alkali-resistant acids with oxygen. Such information will help ensure a maximum yield during subsequent alkaline delignification stages.

As initially reported (1), an effective and versatile reactor was designed and developed for the study of rapid reactions at elevated temperatures and pressures. The reactor could be operated as a flow reactor with reaction times on the order of 0.01 second or as a batch reactor with a maximum reaction time of at least 30 minutes. This versatility was made possible by the discovery that dissolved oxygen could be held in a capillary at elevated temperatures for at least 30 minutes by a back pressure of nitrogen as well as oxygen.

The second report (2) expanded upon problems of reactor design, adopted a method of oxygen analysis based on the Winkler test, and gave reasons for the choice of nickel as the metal used for reactor construction.

The third report (3) targeted cellobiose as a model for the study of the degradation of reducing groups. Preliminary reactions showed that, although some alkali-stable reaction products could be detected, analytical difficulties clouded the interpretations. Specifically, the stability of the several possible aldonic acids was uncertain under the proposed reaction conditions. Since the desired reaction products (glucosylaldonic acids) displayed varying degrees of stability to hot alkali, the reaction of these acids under the proposed conditions must be measured to determine suitable correction factors.

This report covers the development of methods for analyzing reaction products and deals with the reactions of cellobiose and derived aldonic acids with alkali and oxygen. The objective was to determine which of the many possible oxidation products is stable to oxygen and alkali, to determine those parameters which influence their stability, and to determine if these acids can be prepared from cellobiose in significant yields.

The rate of degradation of the acids in oxygen and alkali were in agreement with those inferred from the literature. In particular, glucosylarabinonic acid and glucosylerythronic acid are less sensitive to oxygen oxidation than cellobionic and epicellobionic acids. The overall rate of degradation of glucosylarabinonic acid was the greatest due to its extreme instability in both hydroxide and carbonate solutions in the absence of oxygen. The participation of salts as nucleophiles was discovered to be a factor influencing cellobionic acid degradation and must be incorporated into any statistical program designed to investigate the effects of parameters upon the peeling reaction. The significant degradation of the other acids in sodium carbonate solutions was also consistent with the occurrence of salt effects.

The concentration of alkali used in these experiments was too great for the effective conversion of cellobiose to glucosylaldonic acids. Better results will be achieved using lower concentrations of NaOH and greater oxygen pressures. These results, combined with the results of a long duration low temperature reaction and data in the literature, suggest that, although optimization is possible, alkali-labile arabinonic acid end units will be the most common product unless preferential catalytic methods of oxidations are discovered.

This project has been terminated, and no further active research will be carried out. Related activity in the form of student research and those aspects of polysaccharide instability to alkali that are pertinent to Project 3388 (Non-chlorine Bleaching) will continue.

OBJECTIVE

The removal of lignin during alkaline oxygen delignification does not result in the extensive loss of yield characteristic of many other alkaline pulping processes. The increased yield may reflect the stabilization of cellulosic end groups toward the alkaline peeling reactions. If this is true, pretreatments of carbohydrate material with oxygen to confer alkali stability before conventional alkaline pulping is an attractive possibility, especially since the reactants will not upset existing technological processes. Considerable research has been devoted by others to achieve this goal without much success (4-6). The objective of this project was to determine the effect of different reaction parameters upon the formation of terminal aldonic acid groups in cellulose for the purpose of stabilizing it to the action of alkali.

INTRODUCTION

The mechanisms accounting for the degradation of cellulose by alkali have been extensively studied but not completely elucidated. They include chain cleavage reactions as well as peeling and stopping reactions. The cleavage reactions lead to lower viscosity and poorer strength properties and expose new reducing ends of the molecule to degradations by the peeling reactions. Ultimately the successive peeling stages are stopped by a terminating reaction which in effect replaces the alkali-labile carbonyl group by a relatively alkali-stable carboxyl group.

The mechanisms postulated to occur during the peeling of cellulose by alkali are illustrated in Fig. 1 (6). The rate-determining step in this complex series of reactions is not known, thought to be the formation of a hexulose unit, and is the subject of considerable controversy. The rate determining steps conjectured by Samuelson (7) and Sarkanen (8) are shown in Fig. 2 and 3.

Stabilization to peeling can be achieved if the carbonyl groups of a polysaccharide are reduced (9), oxidized (10,11) or modified by bisulfite formation (12). Reduction confers the greatest stabilization but is best achieved using expensive reagents such as $NaBH_4$ (9). Other modifications such as H_2S pretreatments are dangerous and technologically inconvenient to carry out (13). Oxidation of end groups is less effective but can be achieved using polysulfides without upsetting mill operations greatly. Oxygen oxidation would be more desirable, as the by-products would not upset existing mill operations, and much effort has been spent investigating this possibility.

The analysis of the end groups of cellulose and model substances oxidized by oxygen reported by Samuelson (14) and others (15) demonstrates that gluconic

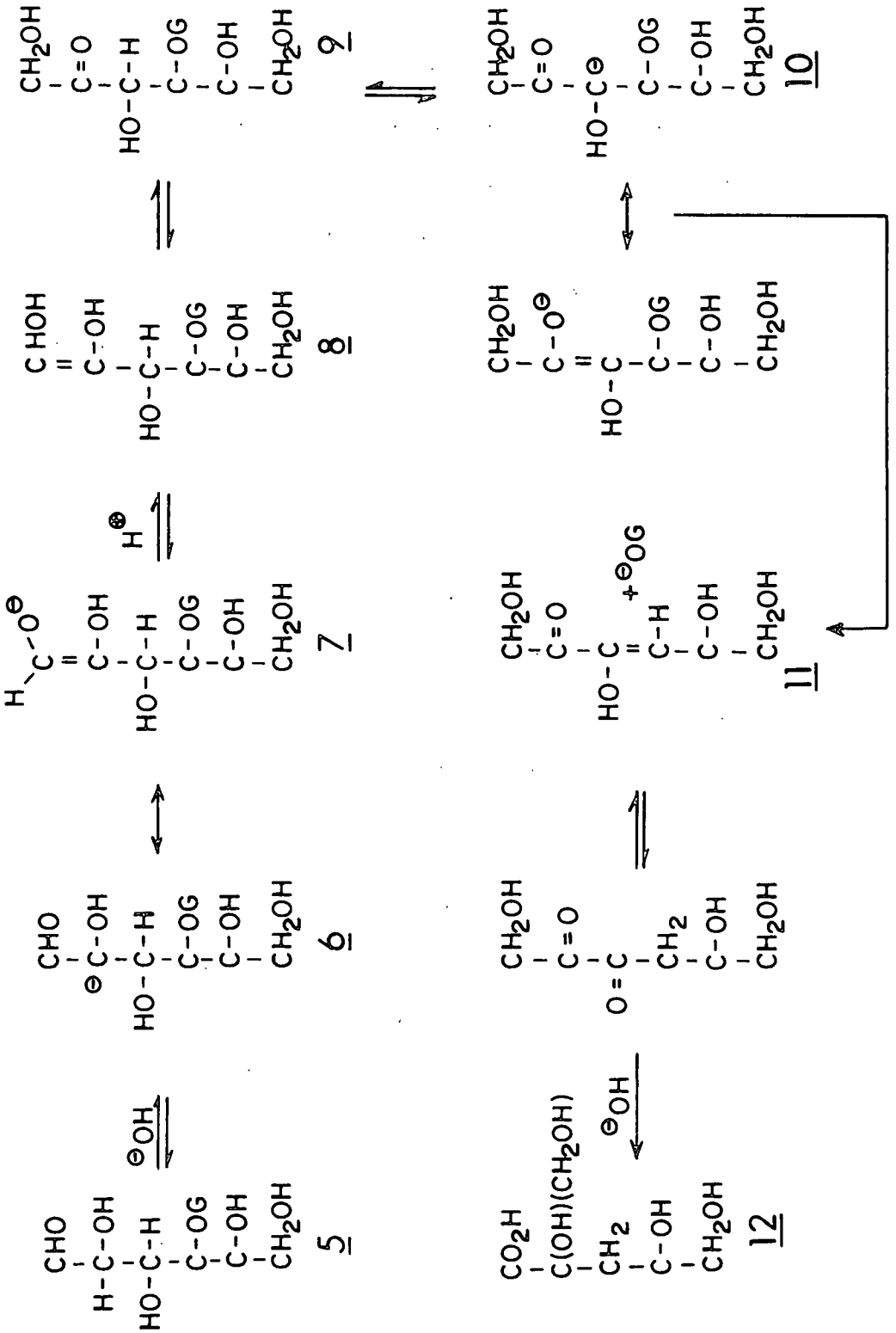

Figure 1. A Possible Pathway for the Peeling Reaction for a 4-O-Substituted Glucose

acid is not a major reaction product. Oxygen does not attack the terminal aldehyde group directly but instead attacks some of the many intermediate alkaline rearrangement products, such as enediols, shown in Fig. 1. A competition for the degradation of terminal end units between alkali and oxygen exists, and the initial interactions of aldehyde and alkali predominate. The major identified acids from oxygen oxidation of cellulose are arabinonic, erythronic, and mannonic acids, while traces of gluconic, ribonic, threonic, and metasaccharinic acids are invariably present (16). As mentioned above, these products result from the oxidation of intermediate species which are formed during the conversion of unmodified carbonyl groups to the various saccharinic acid derivatives.

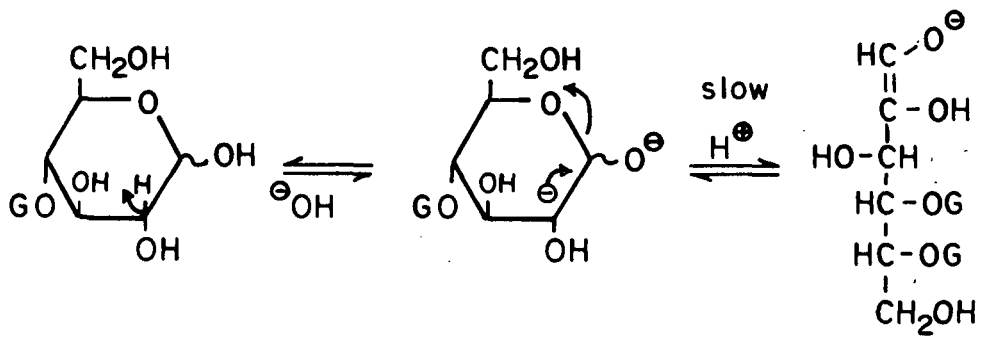

Figure 2. The Rate Determining Step According to Samuelson (7) Leading to the Peeling Reaction

The yield and proportion of the terminating acid groups can be altered by changing the experimental conditions as shown in Table I (14). This observation makes it theoretically possible to devise experimental conditions which will maximize the yield of most alkali-resistant aldonic acids at the reactive sites of the cellulose molecule.

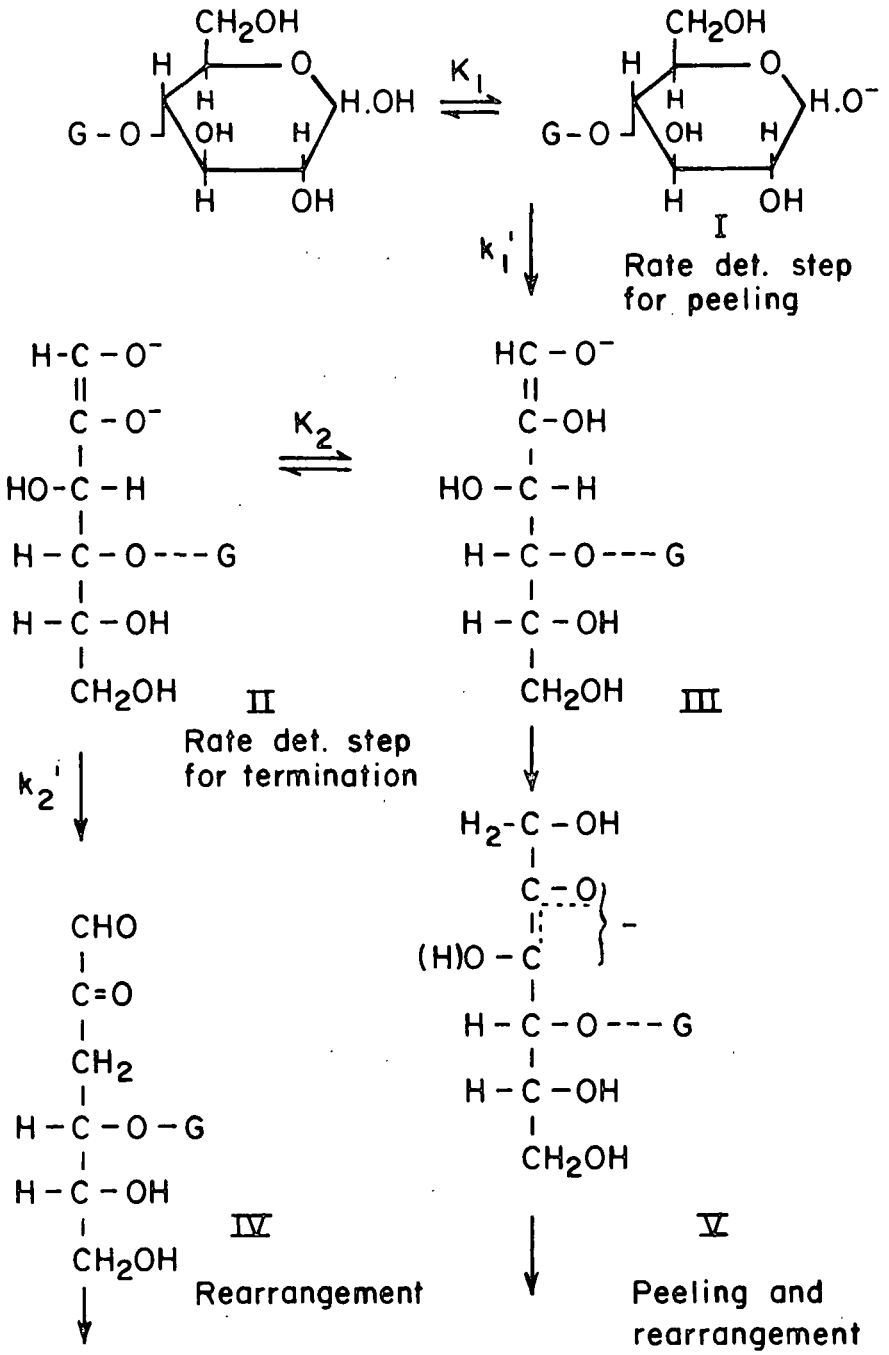

Figure 3. The Rate Determining Step According to Lai and Sarkanen (8) Leading to the Peeling Reaction

TABLE I

THE ALKALINE DEGRADATION OF CELLOBIOSE IN NITROGEN, AIR, AND OXYGEN (17)

Temperature, °C	Atmosphere	Time of Reaction	(0.04N) Base	Combined Yield of Glucosylaldonic Acids[a]
25	N_2	48	$Ba(OH)_2$	0
25	Air	48	"	9.1[b]
25	O_2	48	"	27.1[b]
50	N_2	6	$Ba(OH)_2$	0
50	Air	6	"	3.4
50	O_2	6	"	9.9
100	Air	2	$Ba(OH)_2$	1.5
25	Air	48	NaOH	2.4[c]
50	Air	6	"	1.7
100	Air	2	"	0

[a] These acids were not analyzed separately.

[b] Other experiments have shown this fraction is predominantly glucosylarabinonic acid.

[c] This fraction is not predominantly glucosylarabinonic acid.

The present investigation was begun to investigate the effects of different reaction parameters upon the formation of terminal aldonic acid groups in cellulose by oxygen oxidation. For convenience, cellobiose was chosen as a model since some of its reaction products from the reaction with oxygen in alkaline solutions have already been studied by others (17,18). A statistical program was designed to evaluate the effect of various parameters upon the production of glucosylaldonic acids (19). It was decided not to investigate the known effects of alkaline earth hydroxides and transition metal ions at this early stage of the investigation. The catalytic effects of some of these reagents during oxygen reactions makes future investigation attractive. The obvious parameters such as time, temperature, concentration of oxygen, and pH were studied initially.

The investigation requires a reactor in which reactions of short duration could be carried out at elevated temperatures (between 100 and 130°C) and at elevated oxygen pressures. Convenient techniques were required to be developed for measuring the concentration of reactants and products. Most of the effort spent on this project was directed toward these analytical developments.

The present report has summarized pertinent data from previous reports that have led to the research described here. It is hoped this will give the reader an overall picture of the progress accomplished during this research program.

RESULTS AND DISCUSSION

A brief review of the factors leading to reactor design, construction materials, and the measurement of dissolved oxygen will be given before the discussion of the new results. The reader is referred to the original reports (1-3) for experimental details. This research describes preparation of reactants, measurement of reactants and products, degradation of glucosylaldonic acids in alkaline-oxygen solutions, salt effects, and the reactions of cellobiose with alkali and oxygen.

REACTOR DESIGN

The design of the reactor was based upon experience developed for biological investigations which demanded rapid mixing techniques for the study of reaction times of the order of milliseconds (20,21). Our reactor must be designed to permit reactions at high temperatures, high pH and appreciable oxygen pressures. The reactor developed previously for the study of the peeling reaction (22) was fitted with gas-tight seals and with two chambers for preparing dissolved oxygen and for receiving the liquors after reaction. Special syringes were connected to the pressure chamber to bring solutions of dissolved oxygen and sugar together in the reaction chamber and to provide a quench necessary for the physical isolation and stopping of the reaction. These features are illustrated in Fig. 4.

In operation, reactants A and B are forced simultaneously through a mixer (M) into the reaction zone. The line from this reactor to the receiver is purged of reactants with quench C. After an interval, the solution is forced out of the reaction chamber and is terminated when it reacts with quench (also being forced through the lines) at the second mixer (N). In continuous operation, the reaction time (Ta) is directly proportional to the volume Vc of the reaction zone

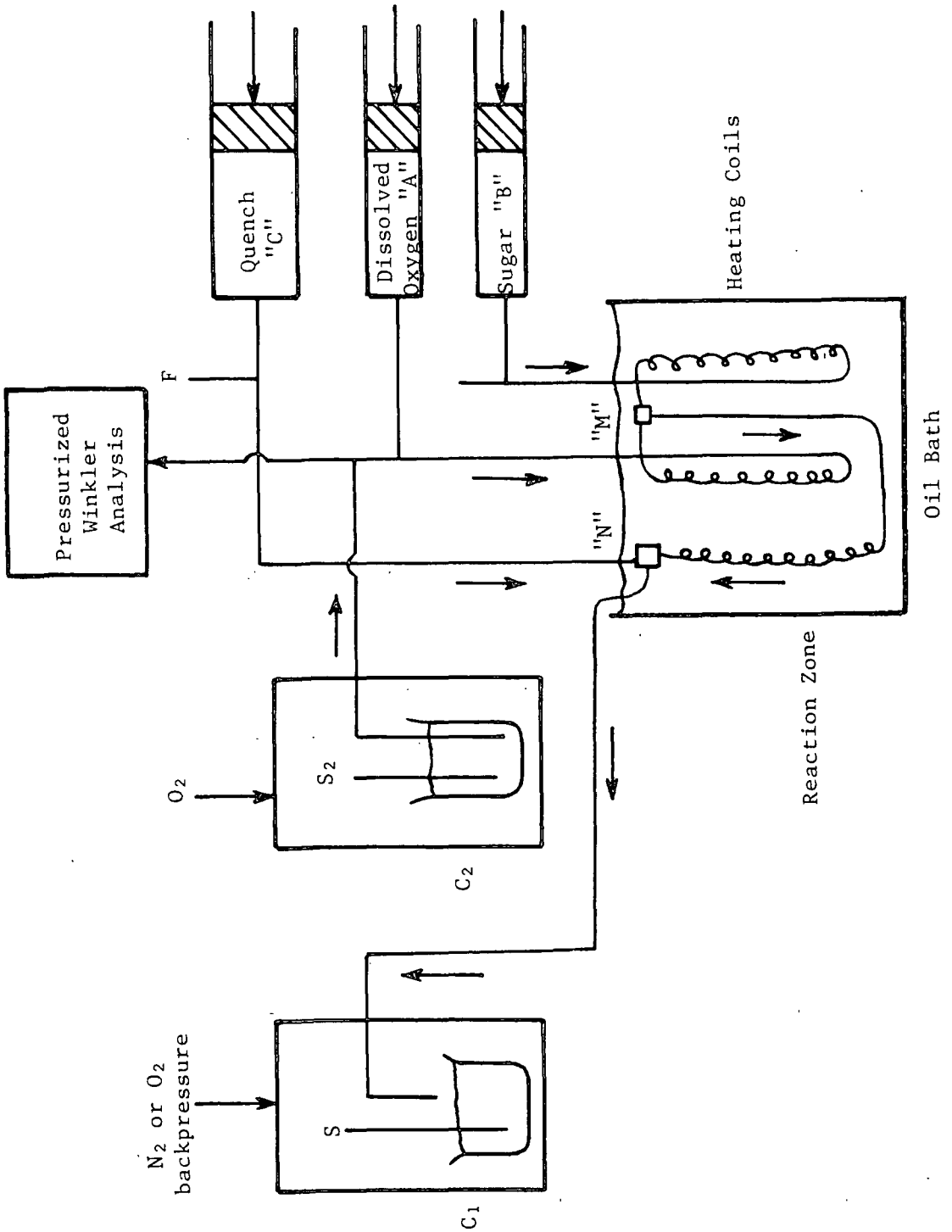

Figure 4. Direction of Flow of Liquids in Flow Reactor

and inversely proportional to the rate of flow of liquid through this zone as follows:

$$Ta = \frac{Vc\ (mL)}{\text{rate of flow (mL/sec)}}$$

The reactor was most frequently operated in a batch fashion during this investigation. Since the dwell time was greatly in excess of the flow times to fill and empty the reactor, no correction factors were required. The operation of the equipment is complicated by the presence of the heating coils between the first mixer to allow time for thermal equilibrium to be established.

The volume of the quenching agent (9) is large compared to the volume of reactants (A and B). It is essential that the latter be bracketed by quenching reagent. This factor is automatically controlled in the apparatus.

CONSTRUCTION OF REACTOR

It was impossible to obtain small bore Teflon or Teflon-lined tubing capable of withstanding the temperatures and pressures necessary for this study. Because of the known catalytic effect of transition metals and their ions on autoxidation reactions of all types (23), it was concluded that the high surface area to volume ratio of stainless steel used in a flow reactor would lead to different results than those obtained from commercial pulping installations with their relatively low surface area to volume ratios. Since the necessary kinetic versatility could not be achieved using other designs incorporating a low surface to volume ratio, it was necessary to change the materials of construction of the existing apparatus design.

The experimental technique necessary for testing metals for their catalytic influence must be capable of predicting their behavior during the reaction of carbonyl

groups of reducing sugars with oxygen. The apparently simpler procedure of testing the effect of reducing sugars themselves was ruled out since the only apparatus capable of handling the reaction was the flow reactor which was being redesigned. An alternative evaluation could be achieved by subjecting test strips of metal to chemical environments in a pressure vessel constructed of stainless steel and lined with Teflon. As mentioned above, the rapidity of the reaction of oxygen and alkali with reducing sugars and the inability to measure the reactants during their short lifetime made their use impossible in this apparatus. Instead, it was conjectured that the evaluation could be conducted more conveniently and inexpensively by reacting methyl glucosides with oxygen and alkali in the presence and absence of test metal strips. Although the glucosides do not initially resemble reducing sugars and, therefore, might not be considered satisfactory models because of the absence of an aldehydic group, their degradation products do go through carbonyl intermediates (24). These ketonic groups are capable of several different reaction pathways, some of which are analogous to the reaction of aldoses with alkali.

The apparatus used for this investigation was the Teflon-lined 1-liter reactor designed by McCloskey (25) and Sinkey (26). It was operated in the manner described elsewhere (27). Test metal strips purchased from Ventron Corp. cut to 1 x 6 inches were attached to the Teflon baffle within the reactor. Methyl-α-D-glucopyranoside, and in a few instances methyl-β-D-glucopyranoside, was reacted with 5% sodium hydroxide and 100 psig oxygen at 120°C for different times in the reactor with the strips present. The reaction was quenched by cooling, and the contents were analyzed for unreacted glycoside by gas chromatography (25). Peroxide analyses for both hydrogen peroxide and organic peroxides were carried out using conventional techniques (28), and the dissolved metal contents of the liquors were measured by flame photometry. The results of these experiments are summarized in Table II.

TABLE II

DETERMINATION OF THE EFFECT OF METALS AND Mg^{+2} ON THE DEGRADATION OF METHYL (α and β) GLUCOSIDES (2)

Metal Tested	Hydrogen Peroxide Formed	Organic Peroxide Formed	Loss of Me (α or β) Glucosides	Effect Metal on Hydrogen Peroxide
None	Normal	Normal	Normal	Normal
Pt	Trace	Small	Induction	Decompose
Ag	Small	Large	--	Decompose
Stainless steel #316	Trace	Large	--	Decompose
Ni	Normal	Normal	Normal	Normal
Zr	Normal	Normal	Induction	Normal
Ti	Normal	Normal	Normal	Normal
W	Large	Large	--	Stabilizing
Co	None	Large	Rapid	Decompose
Mg^{+2}	Large	Small	Induction	Stabilizing

The results indicate that the metals tested, nickel, and possibly titanium and zirconium, alter the degradation of hydrogen peroxide and methyl glucosides in alkali and oxygen at 120°C at the same rate as the control. Other metals have either a stabilizing or a degradative effect on these reactants and would likely affect the degradation of aldoses under similar experimental conditions. It is likely, therefore, that these three metals, and nickel in particular, will make suitable metals for the construction of certain specialized reactors. Proof for this speculation can be obtained only by comparing reactions of glucosidic substances under identical conditions in Teflon and nickel reactors.

MEASUREMENT OF DISSOLVED OXYGEN

Dissolved oxygen was best prepared by spraying water (or electrolytic solution) into a polyethylene bottle contained in an oxygen atmosphere. The amount of oxygen dissolved in water at 26°C was about 80% of saturation, and repeated spraying by recycling oxygenated solutions produced concentrations in excess of 95% of saturation. It is speculated that complete saturation will not be achieved by this apparatus due to the suction action of the piston during the recycling procedure.

Initial attempts to measure dissolved oxygen employed a Beckman Field Lab. oxygen analyzer with a No. 39552 oxygen sensor housed within the saturation chamber. This unit was convenient to operate under atmospheric conditions but was unreliable when used in the pressure chamber. The principal difficulties arose from gas bubbles accumulating around the sensor tip, a loss of calibration due to corrosion within the sensor unit, and the development of short circuits because of moisture penetration and corrosion.

Successful oxygen analysis was achieved by modifying the classical Winkler Wet Analytic scheme for use under pressure. The design of this equipment and its operation are described in Report Two of this project. The reactions involved are as follows, and the liberated iodine can be measured by titration with standard sodium thiosulfate:

$$4MnO + O_2 \xrightarrow{\ominus OH} 2Mn_2O_3$$

$$H_2O + Mn_2O_3 + 2I^{\ominus} \xrightarrow{H^{\oplus}} 2MnO + I_2 + 2OH^{\ominus}$$

The errors in 9 estimations of oxygen concentration were ± 2% of the amount present. The error increased to ± 5% at very low oxygen concentrations. Figure 5 demonstrates

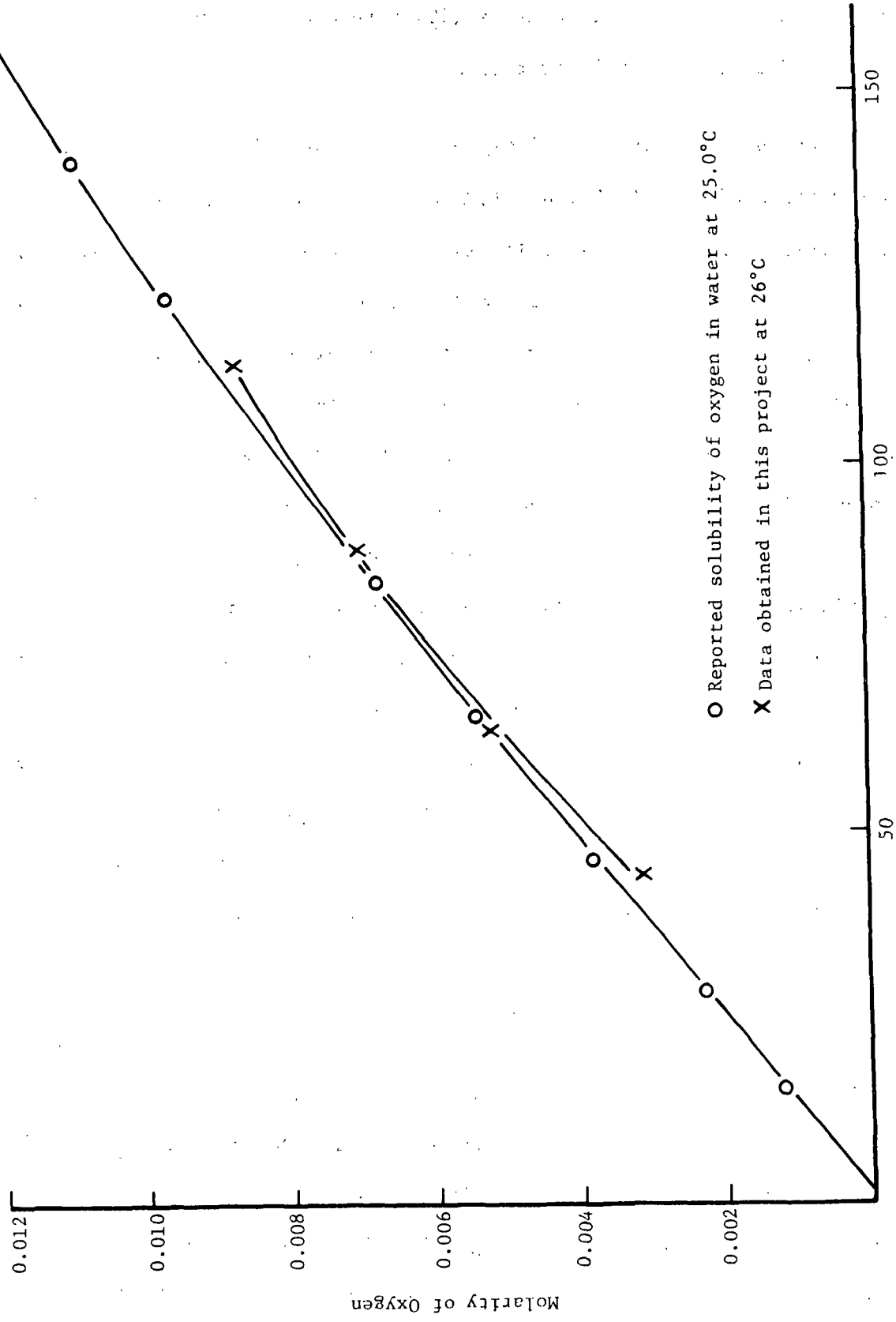

Figure 5. The Solubility of Oxygen in Distilled Water at 26°C

significant agreement with the literature (29) although the results are low due to the difficulty of achieving 100% saturation.

The change in solubility of oxygen in various salt solutions (NaOH, Na_2CO_3) was determined at 0.1, 0.5 and 1.0N concentrations. The behavior of NaOH solutions at these concentrations is in Fig. 6, and the corresponding behavior in sodium carbonate solution is described in Report Two. A comparison of the effects of NaOH, Na_2CO_3, and $NaHCO_3$ on oxygen solubility is given in Fig. 7. At low oxygen pressure, concentration has little effect on solubility in agreement with the literature. At higher oxygen pressures, the effect of NaOH concentrations greater than 0.5N can become increasingly important. This behavior is similar to the published behavior for other salts (30). At atmospheric pressure, the solubility of oxygen in 0.125N solutions of salts of strong acids and bases ranges from 95 to 98% of that found in pure water. In 1N solutions the solubility is about 80% of that found in water. In the present experiments little difference was found in the solubility of oxygen in water or in salt solutions at lower oxygen pressures and salt concentrations. Although the control experiments indicated greater precision, an error of 12% occurs when the oxygen contents of dilute salt solutions are compared to those of water. This might be related to difficulties introduced by surface tension, etc. At higher oxygen pressures and salt concentrations, significant differences of oxygen solubility can occur.

The solubility of oxygen in sodium carbonate and bicarbonate solutions is similar to its reported solubility in ammonium chloride solution (30). In both cases the salt is formed from a weak ion (from a gas dissolved in water) and a strong counterion. The solubility of oxygen in 0.125 and 1.0N ammonium chloride at atmospheric pressure and 25°C is reported to be about 40 and 12% of the solubility in water. The complicating influence of carbonate on oxygen delignification

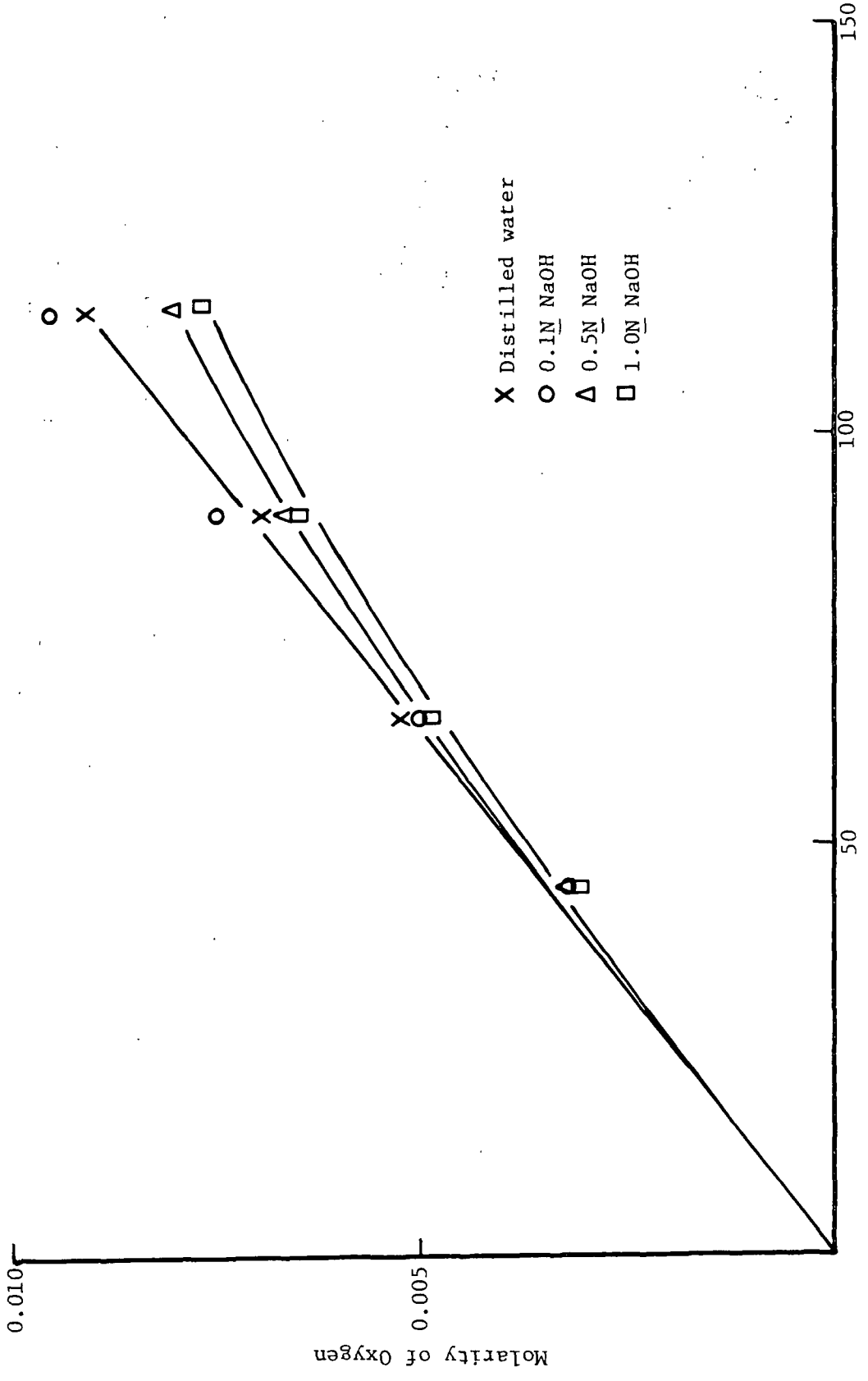

Figure 6. The Solubility of Oxygen in Various Concentrations of Sodium Hydroxide at 26°C

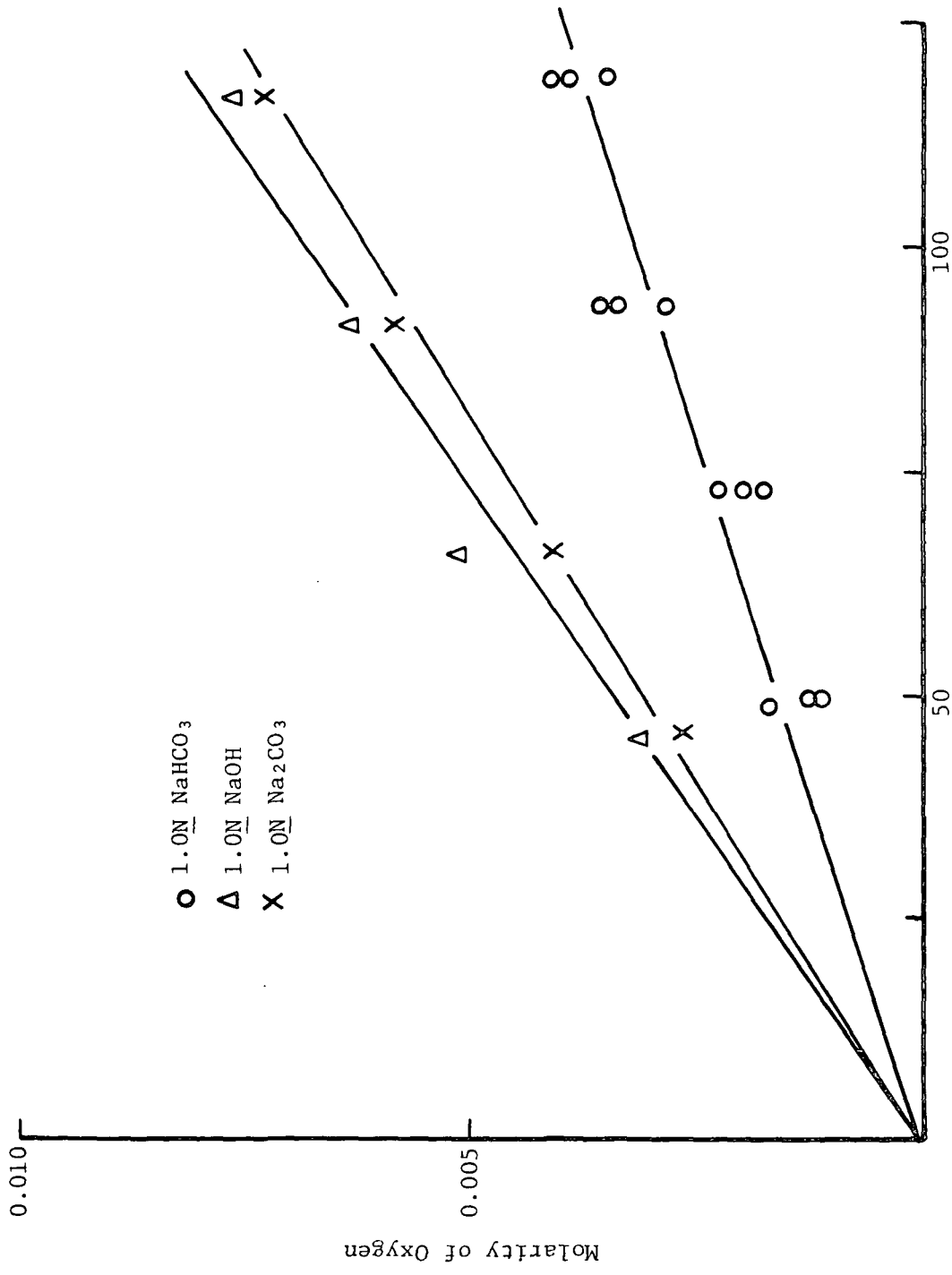

Figure 7. The Solubility of Oxygen in 1.0N Salt Solutions at Different Pressures (Calculated for 26°C)

noted by Samuelson (31) was rationalized by him to be, in part, an oxygen solubility problem, and these data tend to confirm his speculations. Further research at elevated temperatures is needed to prove this trend. The large experimental error indicated for the solubility of oxygen in $NaHCO_3$ solution is real and at present unexplained.

PREPARATION OF REACTANTS

Cellobiose was purchased from Aldrich Chemical Company and used without further purification.

Cellobionic acid was prepared by the scheme developed by Moore and Link (32). This reaction involved the oxidation of cellobiose with KOI and was preferred over other techniques because the reaction products are more easily isolated from unused reactants. This advantage was due to the solubility of all reactants in methanol and the insolubility of potassium cellobionate in both methanol and acetone.

Initial attempts to prepare 3-O-β-D-glucopyranosyl-D-arabinonic acid (or C_{11} acid) by the procedure of Hardegger, et al. (33) were unsuccessful. Analysis of the products of this reaction between cellobiose and oxygen [carried out in $Ba(OH)_2$ solution] yielded a variety of substances from which a small amount of C_{11} acid could be separated as a brucine salt. The procedure was abandoned in favor of a classical Wohl-Zemplin degradation of cellobionitrile acetate (34) to 3-O-β-D-glucopyranosyl-D-arabinose (35). This disaccharide was oxidized with KOI to the potassium salt 3-O-β-D-glucopyranosyl arabinonic acid. The salt was soluble in methanol and could be separated from the reaction mixture by the addition of acetone.

2-O-β-D-Glucopyranosyl-D-erythronic acid (a C_{10} acid) was prepared by subjecting the acetate of 3-O-β-D-glucopyranosyl-D-arabinose to a Wohl-Zemplin degradation and oxidizing the resultant disaccharide to the potassium salt of the C_{10} aldonic acid with KOI. The potassium salt of this acid was soluble in both methanol and acetone and could be separated from most reaction products by trituration of this evaporated reaction mixture with acetone. A small amount of low-molecular weight contaminant included in the extract was not removed by this procedure.

4-O-β-D-Glucopyranosyl-D-mannose was obtained from MacLauren and Green (36). Epicellobionic acid (4-O-β-D-glucopyranosyl-D-mannonic acid) was prepared from the corresponding disaccharide by oxidation with KOI. It was recovered in an identical manner to that used for purifying cellobionic acid.

A summary of the synthetic pathway is given in Fig. 8. The acids were characterized for purity by examining their GLC patterns and their C^{13} NMR spectra. Although assignments were not possible for many peaks, the patterns were consistent with those to be expected from C_{12}, C_{11} and C_{10} acids. The C^{13} NMR spectrum of the C_{10} acid indicated the presence of a trace of carbonyl-containing contaminant.

MEASUREMENT OF REACTANTS AND PRODUCTS

A comparison of the effectiveness of the GLC technique compared to HPLC for the quantitative analysis of reaction products was carried out. The results summarized in Table III indicate the use of GLC is to be preferred if the separation of oxidation products can be improved. The principal drawbacks of the HPLC are high background noise and time-consuming operations.

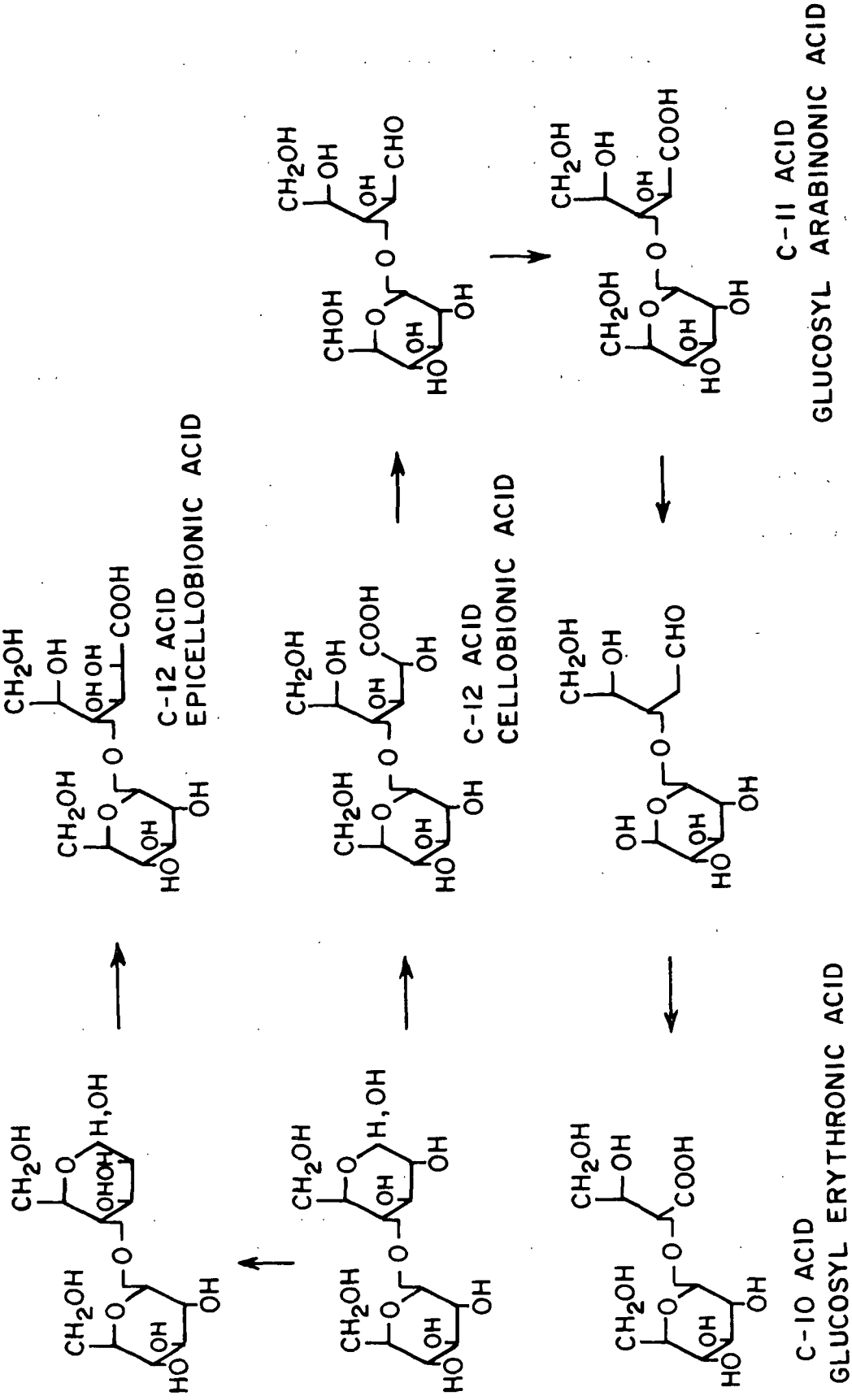

Figure 8. The Synthesis of Various Glucosylaldonic Acids

TABLE III

COMPARISON OF GC VS. HPLC FOR SUGAR ANALYSIS

Relative Sensitivities of Detectors[a]	GC System	LC System
Original sample available for analysis, µg	1000	1000
Derivatization necessary	Yes	No
Sample injected on column, µg	10	200
Sample reaching detector, µg	10	2
Relative percent of original sample	1	0.2
Chemical stability of sample	Poor	Good
Biological stability of sample	Good	Poor
Reuse of sample not reaching detector	No	Yes
Background noise	Low	High
Carbohydrate Separations[b]		
Neutral sugars	Very good	Good
Internal standards	Good	Poor
Neutral and acidic sugars	Poor	Good
Separation according to molecular weight	Good	Poor
Sharpness of peaks	Good	Fair
Time of separations	Good	Fair
Faster movement on column with	Higher temperature	More polar solvents

[a] Both systems use a FID detector, the GC directly, the LC through conversion of nonvolatile carbon to methane on a moving-wire detector.

[b] The two columns compared above are an OV-17 column for a GC system, and a Waters-Bondapak column for an LC system. The sugars have to be derivatized for the GC system.

The oxidation was to be terminated by the addition of 100 mL of a borate buffer solution. This procedure had to be abandoned because subsequent research showed borate complexes formed by this treatment were difficult to decompose without the use of strong mineral acid. Instead, the reaction was to be slowed by cooling and dilution. It was then deionized with Amberlite IR 120-H resin as quickly as possible (less than 5 min). As a result, the alkaline solution was diluted from 0.36N to 0.05N and cooled from 100° to about 30°C. Table IV illustrates the sensitivity of cellobiose to 0.02N NaOH at 60°C and shows the reaction could be slowed sufficiently by the new procedure to act as a quenched reaction. Tests showed all organic reactants could be washed from the IR 120-H resin although this was not the case for anion exchange resins investigated subsequently.

TABLE IV

HALF-LIVES OF REACTIONS OF CELLOBIOSE IN ALKALI-OXYGEN SYSTEMS

System	Peeling Reaction	Stopping Reaction	Oxidation Reaction	Cleavage Reaction
2N Alkali at 170°C	5-10 ms[a]	1000 ms	None	1800 min
Similar, with oxygen	?	?	?	35 min
0.02N Alkali at 60°C	20 min	500 min	None	Very slow
Same for glucose with oxygen at 50°C	Very slow	?	200 min	Very slow

[a] ms = milliseconds.

If the acidic solutions containing glucosylaldonic acids were evaporated directly to dryness, a variety of lactones were formed. The problem was exacerbated by the presence of borate buffers. Typical GLC patterns for the recovery of potassium

cellobionate are shown in Fig. 9. It was concluded the best work-up procedure would involve evaporation of the reaction products from an ammoniacal solution after removal of sodium ion with Amberlite IR 120-H or Dowex AG 50W-X8 resins. The analysis of products from these tests indicated that the sum of the areas beneath the acids and lactones were equal. The area of the acid substances from the borate mixture was not the same as the control, suggesting borate complexes were formed whose response factors differed greatly from those of acids and lactones.

Initial experiments indicated the GLC response of potassium cellobionate (unlike that of inositol and cellobiose) was not a linear function of the sample size and did not even pass through the origin. The trouble was alleviated when the sample port of the GLC was cleaned of carbonaceous debris. Good linear responses passing through the origin depended upon the cleanliness of the sample port and on the removal of degradation products from the column itself. Repeated analysis of sample size vs. GLC response indicated an error of ± 5% when inositol (control) and cellobiose were tested. The error for potassium cellobionate was greater (± 10%) and was probably related to the tendency for the molecule to decompose during GLC analysis. These results suggest a precise kinetic analysis of products is not possible if the quantitative GLC estimations rely upon an internal standard for corrections in sample size variation. The technique will give significant results in a statistically planned program.

Table V illustrates the GLC retention times of TMS derivatives of selected disaccharides and their oxidation and reduction products programmed on an OV-17 column. Although aldobionic acid can be separated from cellobiose, epicellobionic acid cannot be separated. Similarly, the C_{11} and C_{10} acids are not clearly separated from other disaccharides. The interference from reducing substances was eliminated by refluxing a reaction product with 0.36N NaOH for 10 minutes in a

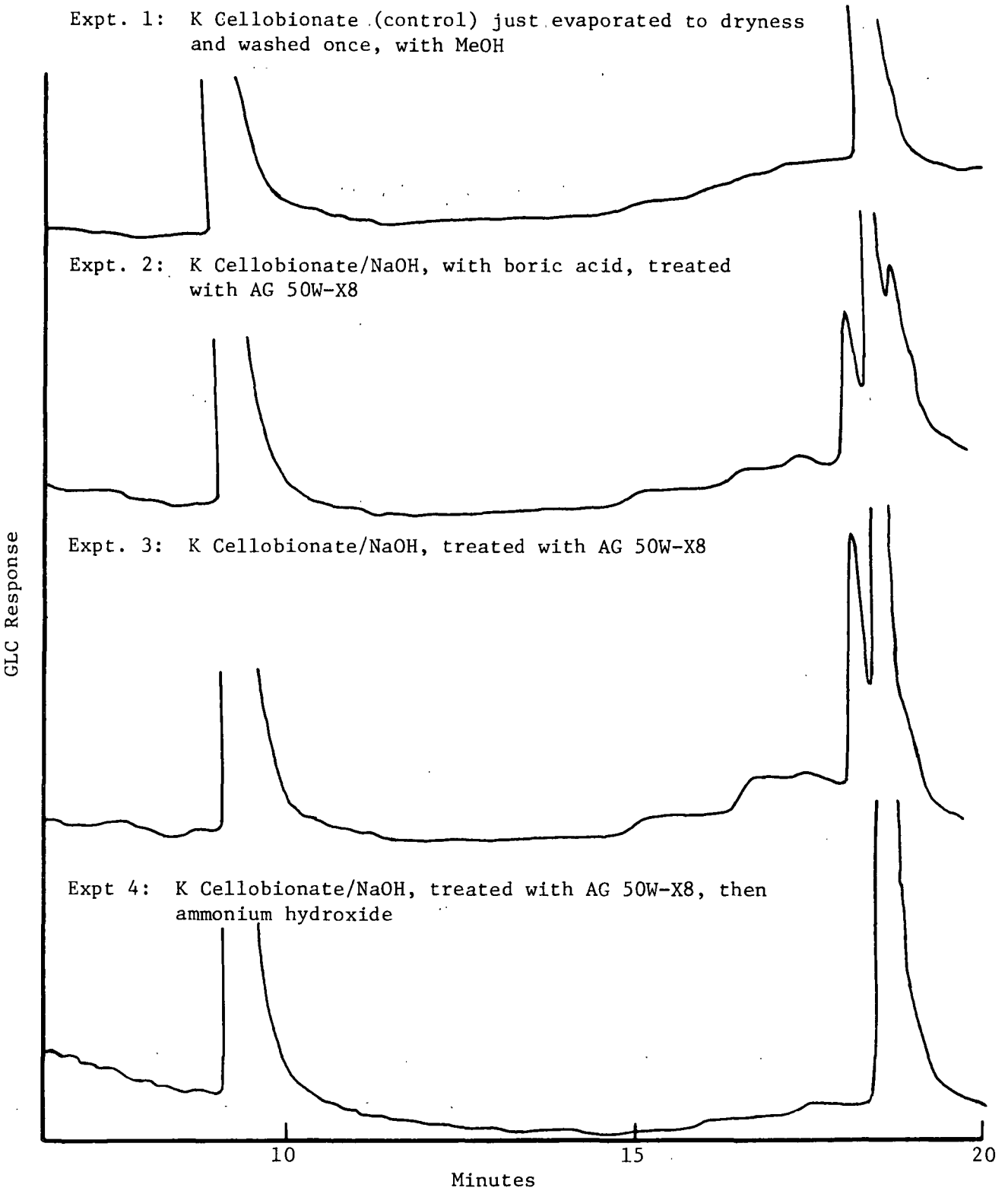

Figure 9. The Effect of Work-up Conditions Upon the GLC Pattern of Potassium Cellobionate

nitrogen atmosphere. Tests showed cellobiose could not be detected after such a test at 100°C, and it was reasoned glucosylaldonic acids would remain for analysis.

TABLE V

RETENTION TIMES OF SELECTED DISACCHARIDES AND THEIR DERIVATIVES[a]

	Sugar, %	Aldonic Acid	Alditol
Cellobiose	19.4[b] 20.5	20.8	20.0
Epicellobiose	19.0 19.8	20.4	19.8
Glucosylarabinose	17.4 18.4	18.7	--
Glucosylerythrose	18.8 20.5	18.6	--
Maltose	--	21.8	--
Inositol (internal standard)	--	--	11.6

[a]Chromatographed on 6 ft x 1/8 inch OV-17 column, programmed from 130-250°C at 6°/min.

[b]Two peaks represent the α and β anomers.

The data in Table V indicate that the separation of C_{11} and C_{10} acids could not be achieved using an OV-17 column. This difficulty was also observed by Malinen and Sjostrom using 3% QF-1 on 80-100-mesh Chromosorb W-HMDS (5). It was planned to resolve this difficulty in several ways if suitable GLC conditions, derivatives, or columns could not be found to effect separations. Acid hydrolysis of the mixture would liberate gluconic, mannonic, arabinonic and erythronic acids which can be separated by GLC. An alternative, but less attractive, procedure would involve a more drastic alkaline degradation to destroy the alkaline-labile

glucosylarabinonic acid in preference to the C_{12} and C_{10} acids. Knowledge of the relative stabilities of glucosylaldonic acids to alkaline degradation is, therefore, necessary for the development of satisfactory analytical schemes.

Unexpected analytical difficulties arose because of the catalytic influence of silica during sample analysis. The stability of potassium cellobionate to alkaline degradation was tested by refluxing it in Pyrex glassware under a nitrogen atmosphere for periods of time up to 30 minutes. It was hoped to derive a correction factor which could be applied to compensate for losses when a reaction sample was similarly treated to destroy residual cellobiose. After 5 minutes the yield of acid was 1.7% and was negligible after 10 minutes. This behavior did not agree with that in the literature. The missing potassium cellobionate was found to be associated with a small quantity of water insoluble siliceous residue in an evaporation flask. Proof consisted of elemental analysis of the residue, colorimetric tests for cellobionate ion (37), and the GLC pattern obtained from silylation of the water insoluble residue.

The oxygen-free alkaline degradation of potassium cellobionate under identical conditions in the nickel coils of the isothermal digester gave more significant results shown in Fig. 10. In one experiment, caustic which had been stored in polypropylene containers, but which had been boiled in Pyrex equipment to expel CO_2, still gave abnormally low results. Only the caustic which had been prepared, stored and treated in nonsiliceous containers gave results comparable to those reported in the literature for the degradation of potassium cellobionate.

This interaction of cellobionic acid — and possibly all degradation products containing an acidic group — may account for the inability to detect glucosylmetasaccharinic acid following degradation of cellobiose with alkali. The more complex spectrum of degradation products, observed from the reaction of silica-free cellobiose with alkali, supports this contention.

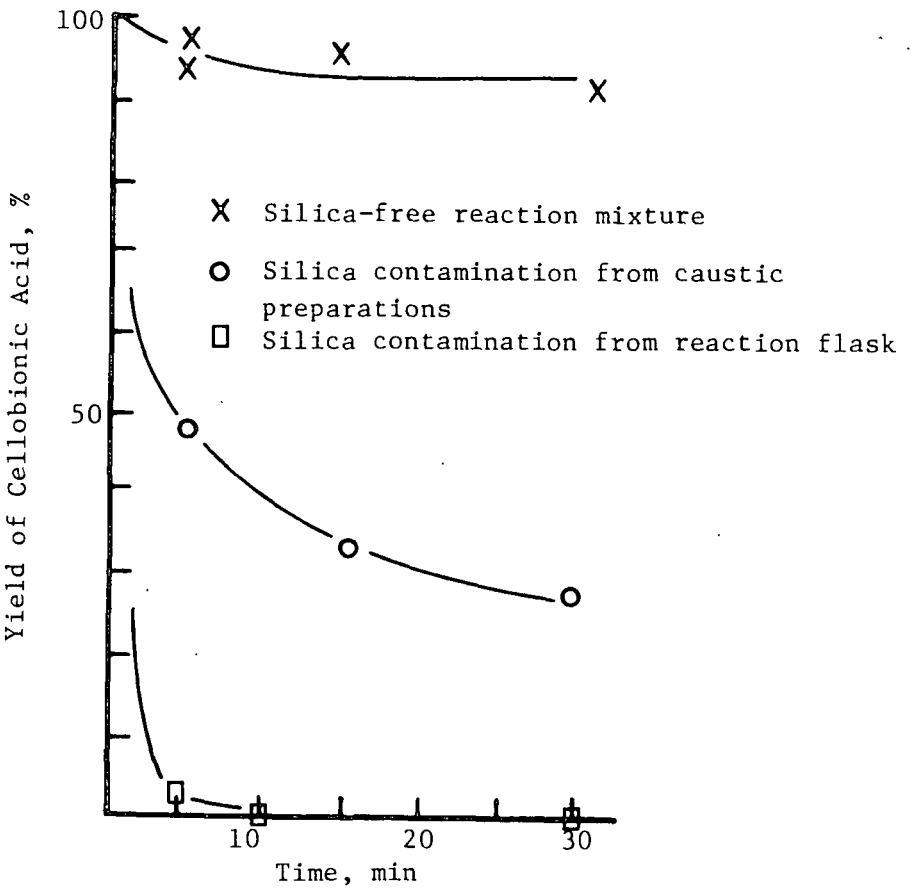

Figure 10. The Reaction of Potassium Cellobionate with Oxygen-free 0.36N NaOH at 100°C in N_2 Atmosphere

THE DEGRADATION OF GLUCOSYL ALDONIC ACIDS IN ALKALINE SOLUTIONS

The loss of potassium cellobionate at 100°C in 0.36N NaOH and 0.36N Na_2CO_3 solution in the presence and absence of oxygen was determined after 5, 15, and 30 minutes reaction and is shown in Fig. 11. In the absence of oxygen, degradation is greater in NaOH than in Na_2CO_3. The degradation is greater in the presence of oxygen than in its absence and is significantly greater in NaOH solution than in Na_2CO_3 solution, suggesting the importance of pH to autoxidation in this instance. The reaction in NaOH is consistent with the results reported by Malinen and Sjostrom (6), where about 88% cellobionic acid was retained after a milder oxidation. The reaction with oxygen in the presence of Na_2CO_3 was significant and informative as

to the nature of the autoxidation reactions. No evidence for epicellobionic acid was observed in the reaction products in agreement with the literature (5,6).

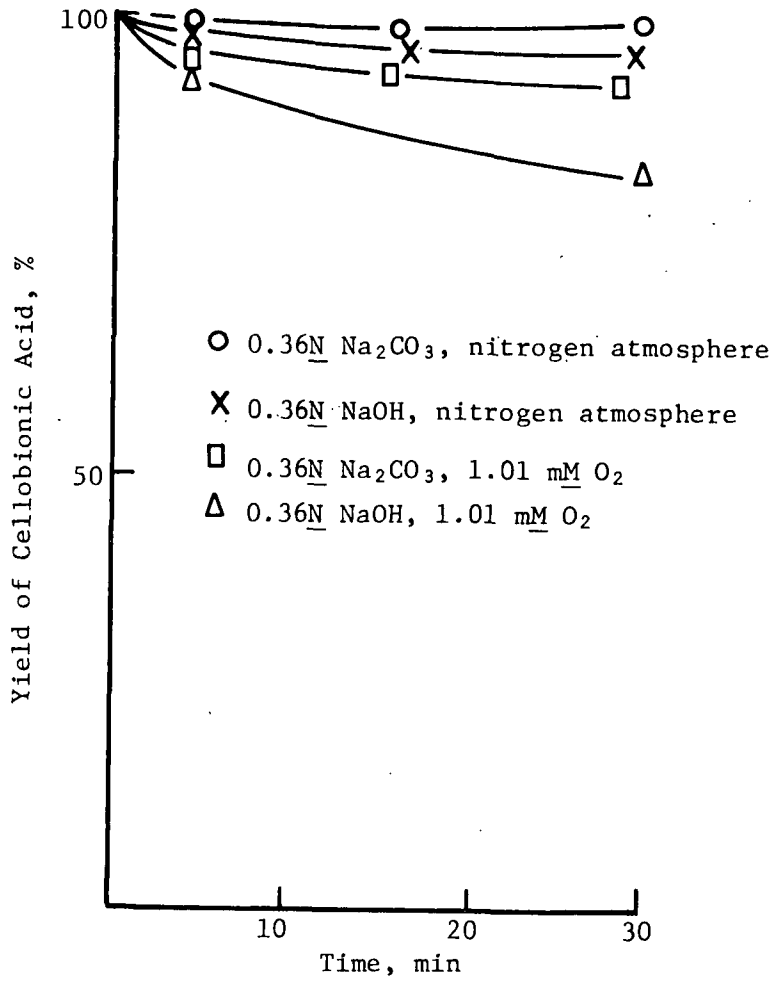

Figure 11. The Reaction of Potassium Cellobionate at 100°C with 1.01 mM Oxygen in 0.36N Na_2CO_3 or 0.36N NaOH

The data in Fig. 12 illustrate that epicellobionic acid reacts with oxygen in both NaOH and Na_2CO_3 solutions at a slightly greater rate than cellobionic acid under the conditions employed here. The greater tendency toward epimerization exhibited by mannonic acid in alkali compared to gluconic acid (38) may be related to the greater activity toward alkali of epicellobionic acid compared to cellobionic acid. Although there is little difference between the effect of NaOH and Na_2CO_3

on the degradation of epicellobionic acid indicated in Fig. 12, this difference is not obscured by experimental error. No evidence for cellobionic acid was observed, and the patterns of the GLC suggested that the aglycone part of epicellobionic acid, like that of other glucosylaldonic acids, degraded to low molecular weight acids.

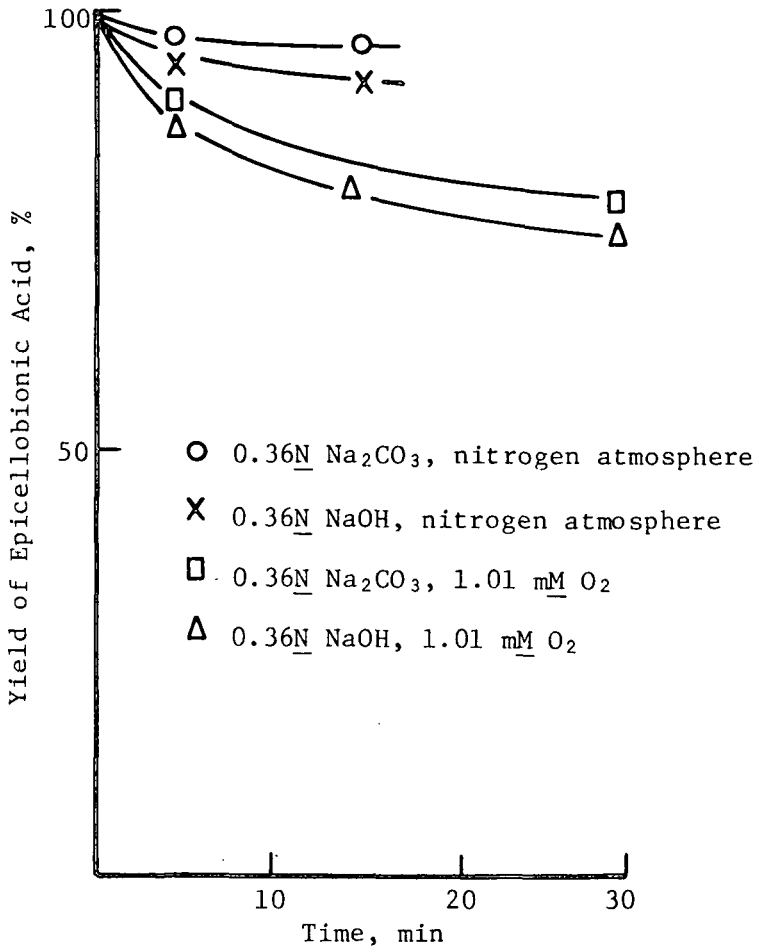

Figure 12. The Reaction of Potassium Epicellobionate at 100°C with 1.01 mM Oxygen in 0.36N Na_2CO_3 or 0.36N NaOH

Plots comparing the degradation of glucosylarabinonic acid at 100°C in 0.36N NaOH and Na_2CO_3 in the presence and absence of oxygen are given in Fig. 13. The analyses of these degradations were very erratic compared to those of the

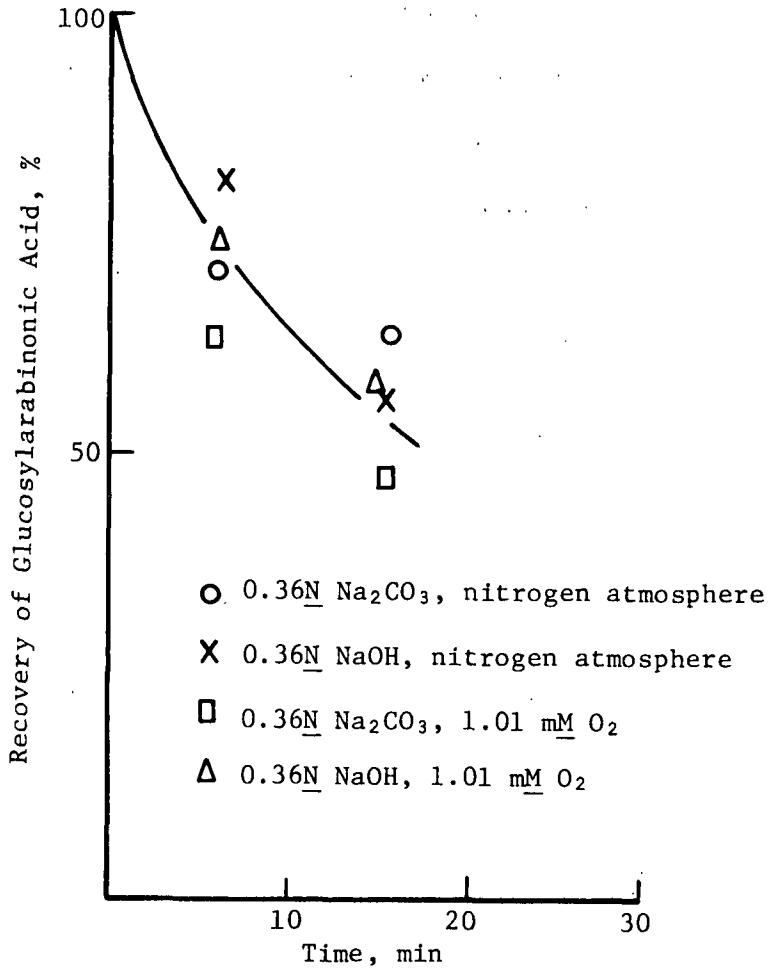

Figure 13. The Reaction of Potassium Glucosylarabinonate at 100°C with 1.01 mM Oxygen in 0.36N Na_2CO_3 or 0.36N NaOH

previous degradations. The trends exhibited in Fig. 13 confirm evidence in the literature (5,39) demonstrating that this acid is less resistant to alkaline degradation than cellobionic acid. Despite the erratic results, little difference exists between the degradation in NaOH and in Na_2CO_3. This lack of difference is thought to be meaningful. Similarly, the lack of difference between the effect of oxygen and its control is thought to be meaningful. Malinen and Sjostrom found glucosylarabinonic acid to be only slightly more rapidly degraded by oxygen in NaOH than in NaOH alone. The loss of aldonic acid observed here is greater than

would be expected from the data of Malinen and Sjostrom. Those researchers observed a 97% recovery when glucosylarabinonic acid was reacted at 120°C for 90 min in 0.025N NaOH (pH 12.25). In this research, only 65% of the original acid could be recovered after reacting for 15 minutes at 100°C in sodium carbonate solution (pH 11.25). This difference is greater than the experimental uncertainties and may be attributed to the greater salt concentrations employed here.

The degradation of glucosylerythronic acid under the conditions described here support the observations of Malinen and Sjostrom (5,6) which indicate it is not as reactive as glucosylarabinonic acid. Szabo and Teder (39) found this acidic end group to be as sensitive to alkali as the arabinonic acid end group. The plots in Fig. 14 demonstrate that oxygen contributes little to the degradation of glucosylerythronic acid in these experiments. Malinen and Sjostrom (5,6) found this acid to be degraded at a slightly greater rate in the presence of oxygen and alkali than in the presence of alkali alone. The greater alkali concentration employed here may contribute to this small difference.

A summary of the behavior of the glucosylaldonic acids to degradation by oxygen in the presence of 0.36N NaOH or 0.36N Na_2CO_3 is given in Table VI. The comparison suggests that different degradative pathways exist for the reaction of alkali and oxygen and are dependent upon molecular configuration. For example, increasing the number of hydroxyl groups between the carboxyl group and the glucosyl unit increases the effect of oxygen on degradation. This might occur as a result of the increased opportunity for the removal of hydrogen as shown below:

The degradation of the molecule would then follow pathways already elucidated at the Institute (25-28,40) for the degradation of polyhydroxy substances. In the case of

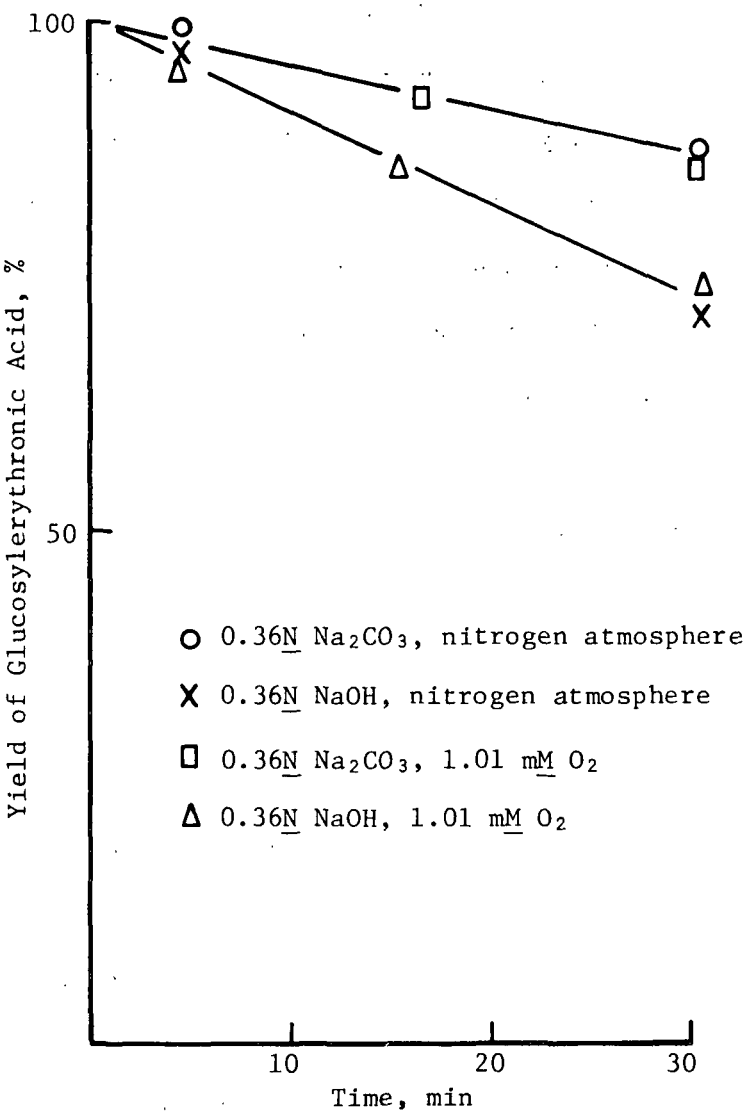

Figure 14. The Reaction of Potassium Glucosylerythronate at 100°C with 1.01 mM Oxygen in 0.36N Na_2CO_3 or 0.36N NaOH

TABLE VI

SUMMARY OF THE BEHAVIOR OF GLUCOSYLALDONIC ACIDS TO OXYGEN SODIUM HYDROXIDE AND SODIUM CARBONATE

	Relative Rate of Degradation	NaOH vs. Na_2CO_3	O_2 vs. N_2
Cellobionic acid	4	NaOH faster	O_2 significantly greater
Epicellobionic acid	3	NaOH faster	O_2 significantly greater
Glucosylarabiononic acid	1	Little difference	Little difference
Glucosylerythronic acid	2	NaOH faster	Little difference

glucosylarabinonic acid, the lability of the glucosyl leaving group beta to the carboxyl unit predominates over hydrogen abstraction (41-43). This same reaction may initiate the limited instability of C_{12} acids in oxygen-free alkali. Because hydroxyl is not a good leaving group (compared to the glucosyl unit) and possibly because subsequent elimination reactions are necessary to effect cleavage, the C_{12} acids are not as sensitive to alkali as the C_{11} acids. Glucosylerythronic acid has no hydroxyls between the carboxyl and the glucosyl unit. As a result of the above conjectures oxygen has little effect on its degradation in alkali. The degradation probably depends entirely on an initial attack on the β position to the carboxyl.

SALT EFFECTS

The results obtained here, compared with those in the literature, suggest that other factors than pH, oxygen concentration, and temperature are involved in the degradation of glucosylaldonic acids. This conjecture was examined by reacting potassium cellobionate with 0.36N sodium acetate at 100°C in the presence and absence of oxygen. In the absence of oxygen, no reaction was evident, while in the presence of 1.26 mM dissolved oxygen, potassium cellobionate was degraded to the same extent as in sodium carbonate solution, and almost as rapidly as in 0.36N NaOH and 1.01 mM oxygen (Fig. 15). The degradation of cellobionic acid is even greater in more concentrated acetate solutions. The effect of salt solutions upon the degradation of cellobionic acid with oxygen raises the unanswered question of what is its effect in actual pulping processes. The other glucosylaldonic acids are probably equally sensitive to the presence of salts. This factor may explain the contradicting data in the literature concerning the stability of these acids in alkaline solutions. Further confirmation of this speculation would be achieved by conducting reactions in the presence of bases and/or salts composed of

progressively changing nucleophilicity and basicity. The use of monovalent salts of halogens might distinguish between the effects of activity and nucleophilicity.

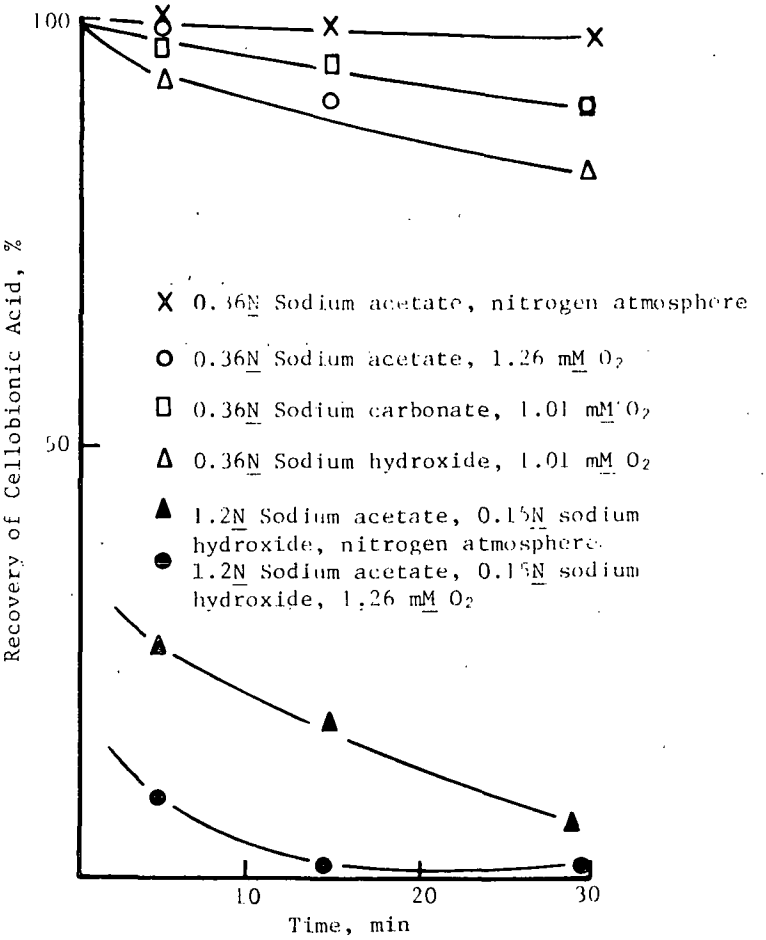

Figure 15. The Reaction of Potassium Cellobionate with Oxygen at 100°C with 0.36N Salt Solutions

REACTIONS OF CELLOBIOSE WITH ALKALI-OXYGEN

Many preliminary reactions of cellobiose with NaOH and Na_2CO_3 were carried out. The half-life of cellobiose in 0.36N NaOH at 100°C of about 10 seconds (and about 40 seconds in 0.36N Na_2CO_3) is consistent with the data given earlier by Green (22) at 65°C and 170°C. Unfortunately, all analyses of glucosyl-aldonic acid as a result of the reaction of cellobiose with alkali and oxygen are subject to error. The error is the result of losses due to resin sorption, changes

in the GLC degradation pattern due to lactone formation, as well as to cross esterification (leading to abnormally large apparent glucosylaldonic acid responses), complications caused by borate complexes, and to the effects of silica contaminants. These sources of error are now recognized, and future analysis will benefit from this knowledge.

A reaction of cellobiose with 0.36N NaOH and 0.36N Na_2CO_3 at three temperatures and in the presence and absence of oxygen was carried out. The results indicated that the reaction of reducing groups with alkali predominated, and very little oxidation of cellobiose to glucosylaldonic acid derivatives occurred. Yields of the acids, though still very small, were greater in the case of carbonate solutions. These data cannot be compared with those in the literature, since these are the first attempts at isothermal reactions — all other results have heat-up periods frequently greater than actual reaction times. Nevertheless, it can be concluded that an increase in oxygen pressure is beneficial for acid production, and optimum reaction times, pH, and temperatures exist which can maximize aldonic acid yield at a given salt concentration and oxygen pressure.

The existence of radical reaction pathways cannot be discounted during the reaction of cellobiose with oxygen and alkali. The existence of hydrogen peroxide reaction intermediate was demonstrated earlier (2), and the unusual degradation of cellobiose-derived aldonic acids during prolonged room temperature autoxidations support this conjecture. Sources of radicals, as can be found during oxygen delignification, can therefore have an influence on both the formation and the stability of the aldonic acid end groups of polysaccharides. The study of radicals on the chemical characteristics of pulps will be explained in future projects at IPC.

CONCLUSIONS

The oxidation of cellulosic end groups to aldonic acids will confer some resistance to peeling during normal alkaline pulping processes. This stabilizing action will be least for arabinonic (and other C_5 acid) end groups. Although preferred terminal aldonic acids would be gluconic or mannonic acids, the preferential oxidation of end groups to these acids has not yet been achieved. Erythronic acid end groups (C_4 acids) would be less satisfactory than mannonic acid at high pH and would be approximately equivalent at lower pH.

The mechanisms by which the aldonic acids are destroyed by alkali are complex and related to the position of the aglycone to the carboxyl group, the number and orientation of hydroxyl groups, pH, and the presence of salts. The latter factors suggest low consistency processes should be more favorable than high consistency processes and should confer greater yield retention. The degradation in oxygen is facilitated by the presence of increasing numbers of hydroxyl groups between the carboxyl and the glycosidic bond.

The alkali concentration employed during preliminary oxidations of cellobiose was too great for the formation of significant yields of glucosyl-aldonic acids. Nevertheless, these data indicate greater yields can be achieved by decreasing alkalinity and salt contents and increasing oxygen pressure. Most of the acids formed in the preliminary experiments were the undesirable glucosyl-arabinonic acids.

Therefore, yield losses cannot be totally prevented by preliminary oxidation of carbohydrate end groups to aldonic acids. This rationalization results not only from the relative instability of terminal aldonic acids themselves but mostly

because the oxidative processes themselves will introduce new end groups into cellulose as a result of chain cleavage. The relatively slight yield loss observed as a result of oxygen delignification compared to other alkaline processes is probably the result of the rapid loss of pH and the relative stability of reducing groups themselves to buffered solutions.

FUTURE ACTIVITY

No further work under this project is planned. However, the effect of salt concentration (ionic strength, nucleophilicity?) on the peeling stopping reactions and the oxidation reactions, together with explorations of desirable catalytic pathways, offer possibilities for future research.

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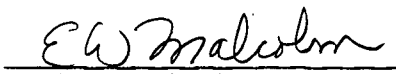

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